Known plant DNA repair and recombination pathways are not important for T-DNA integration

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Previous work from several laboratories implicated double-strand DNA (dsDNA) breaks as important for T-DNA integration into the host genome. Although T-DNA preferentially targets induced dsDNA breaks for integration, the importance of host dsDNA break repair and recombination proteins during integration remains controversial. Differing transformation frequencies among *Arabidopsis* and rice DNA ligase IV (*ligIV*), *xrcc4*, and *ku70/80* mutants have been reported. Somewhat surprisingly, results from the Gelvin, Mysore, West, and Zhang laboratories indicated that mutation/downregulation of *XRCC4, Ku70, Ku80*, and DNAligase VI (*LigVI*) increased transformation susceptibility. We interpret these results to mean that slowing the DNA break repair process provides more opportunity for T-DNA to integrate. Overexpression of *Ku70* or *Ku80* does not decrease transformation susceptibility, suggesting that these proteins are already present in saturating amounts in wild-type plants. Recently, Charles White's laboratory published that simultaneous mutation of genes involved in multiple DNA repair and recombination pathways results in decreased stable, but not transient, transformation. We obtained these mutants and conducted quantitative transient and stable transformation assays. These mutants have major growth defects, especially under high light conditions, which likely reflect accumulated mutations in these plants. Plants grown under both high and low light conditions have greatly decreased transient transformation susceptibility. Surprisingly, their stable transformation susceptibility is not greatly decreased in both growth conditions. Considering the low transient transformation results, our data suggest that stable transformation increases in these mutants, consistent with our hypothesis that decreasing the efficiency of dsDNA break repair increases T-DNA integration. Using the formation of T-circles as a surrogate for T-DNA integration, we show that neither the efficiency of T-circle formation nor their T-DNA border junction characteristics are altered in a *ku80 Arabidopsis* mutant. Taken together, these results are consistent with our hypothesis that known DNA repair and recombination pathways are not essential for T-DNA integration. However, double-strand DNA breaks are likely important for T-DNA integration because RNAi inhibition of both *Arabidopsis* H2A.X genes (*HTA3/5*) greatly decreases stable transformation.